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GALENIC FORMULATIONS

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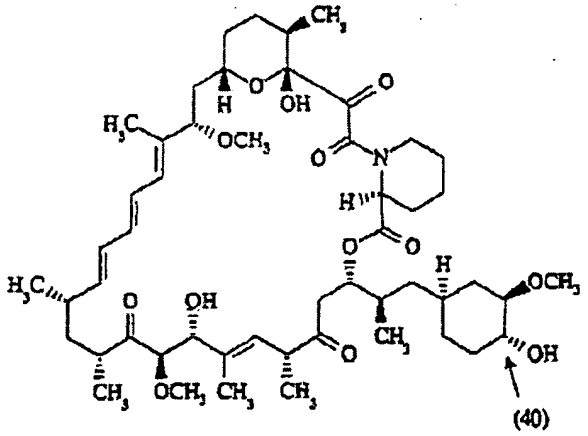
Abstract

Pharmaceutical composition containing a macrolide, e.g., a rapamycin compound, in an emulsion preconcentrate or microemulsion preconcentrate for oral administration. The carrier for the rapamycin compound includes a hydrophilic phase, a lipophilic phase and a surfactant. The composition is stable and furnishes high absorption efficiency.

The present invention pertains to galenic formulations containing macrolides, e.g., compounds of the rapamycin class. The present invention pertains, in particular, to galenic formulations that are in the form of microemulsions, microemulsion preconcentrates, emulsions, or emulsion preconcentrates.

The macrolide can contain 1, 2 or 3 ring oxygen or nitrogen or other atoms, in addition to carbon atoms. It can have side chains, e.g., in the form of fused rings, or substituents, e.g., hydroxy groups. It can contain double bonds. It can contain from 15 to 35 ring atoms, e.g., carbon.

Rapamycin is a macrolide antibiotic prepared from *Streptomyces hygroscopicus*. It has been found that it is useful in a number of applications pharmaceutically, especially as an immunosuppressant, for use in the treatment and prevention of organ transplant rejection and autoimmune diseases. Rapamycin has the following structure:



(Kessler, H., et al., Helv. Chim. Acta (1993) 76:117; U.S. Patent No. 3,929,992). Large numbers of rapamycin derivatives have been synthesized, including those described in U.S. Patent Nos. 5,221,670 and 5,221,740, specific acyl- and aminoacrylrapamycins (see, e.g., U.S. Patent No. 4,316,885, and U.S. Patent No. 4,650,803 and U.S. Patent No. 5,151,413), and carbonates and amide esters (see, e.g., EP 509795 and 515140), 27-desmethylrapamycin (see, e.g., WO 92/14737), 26-dihydrorapamycin (see, e.g., U.S. Patent No. 5,138,051), alkoxyester

derivatives (see, e.g., U.S. Patent No. 5,233,036) and certain pyrazole derivatives (U.S. Patent No. 5,164,399).

Rapamycin and its structurally similar analogues and derivatives are collectively referred to as "compounds of the rapamycin class" in this description.

Compounds of the rapamycin class are very strong immune suppressants, and also have anti-tumor and anti-fungal activity. Their use as pharmaceuticals, especially in oral administration, is restricted by their low solubility, low and variable bioavailability and high toxicity. Little is known concerning the causes of these properties and the absorption site. It can therefore be assumed that low bioavailability occurs, because of extensive metabolism of the macrolide ring, and it cannot be released from a galenic formulation. An acceptable pharmaceutical composition, containing compounds of the rapamycin class, is therefore required.

FK506 is a macrolide immune suppressant produced from *Streptomyces tsukubaensis* No. 9993. The structure of FK506 is given in the Appendix of the Merck Index as A5. A large number of related compounds are also known that include the fundamental structure and immunological properties of FK506. These compounds are described in a large number of publications, e.g., EP 184162, EP 315973, EP 323042, EP423714, EP 427680, EP 465426, EP 474126, WO 91/13889, WO 91/19495, EP 484936, EP 532088, EP 532089, WO 93/5059 and the like. Little is known about the biopharmaceutical properties of such components. These components are referred to in this description collectively as "FKF506 [sic; FK506] compounds."

It has now surprisingly been found that stable compositions containing macrolides that offer high absorption efficiency can be obtained, if the macrolides are formulated with specific carriers.

The present invention therefore furnishes a pharmaceutical composition that includes a macrolide and a carrier that comprises a hydrophilic phase, a lipophilic phase and a surfactant.

In another respect, the invention furnishes a pharmaceutical composition that includes an orally administrable active ingredient that is different from a cyclosporin, and a microemulsion preconcentrate carrier for it, which includes the following:

i) a reaction product of castor oil and ethylene oxide,

ii) a transesterification product of a vegetable oil and glycerol, comprising mostly mono- di- and triglycerides of linoleic acid or oleic acid or a polyoxyalkylated vegetable oil,

iii) 1,2-propylene glycol and

iv) ethanol.

The pharmaceutical composition is stable and is obtained in surprisingly high and persistent absorption efficiency if administered orally. The macrolide could therefore be administered in lower doses, which reduces toxicity problems; e.g., animal experiments in which

the pharmaceutical compositions were administered orally showed high bioavailabilities. The pharmaceutical compositions therefore have very surprising properties that offer significant advantages.

The composition is preferably furnished in the form of a "microemulsion preconcentrate," or "emulsion preconcentrate," especially a form that produces O/W (oil-in-water) microemulsions or emulsions. The composition, however, can be in the form of a microemulsion or emulsion that additionally contains an aqueous phase, preferably water.

A "microemulsion preconcentrate" is defined in this description as a formulation that spontaneously forms a microemulsion in an aqueous medium, e.g., in water or in gastric juices, after oral administration.

A "microemulsion" is a non-opaque or essentially non-opaque colloidal dispersion that is spontaneously or essentially spontaneously formed when its components are brought into contact with each other. A microemulsion is thermodynamically stable and contains scattered particles smaller than about 2000 Å. Microemulsions generally include droplets or particles with a diameters less than about 1500 Å, typically from 30 to 1000 Å. Additional features can be found in the British Patent Application 2 222 770 A, whose description is incorporated here by reference.

An "emulsion preconcentrate" is described in this description as a formulation that spontaneously forms an emulsion in aqueous medium, e.g., in water or in gastric juices, after oral administration. The emulsion formed is opaque, thermodynamically stable and contains scattered droplets with a size greater than about 100 nm, usually greater than about 200 nm. Bimodal size range distributions are frequently obtained. The emulsion preconcentrates are preferably of the type that produce O/W (oil-in-water) emulsions.

A "pharmaceutical composition" means a composition in which the individual components or ingredients are pharmaceutically acceptable themselves and, if a special form of administration is prescribed, are suitable or acceptable for this type of administration.

The lipophilic phase can include 10 to 85 wt% carrier; preferably 15 to 70 wt%, more preferably 20 to 60 wt%, and even more preferably about 25 wt%.

The surfactant can comprise 5 to 80 wt% with a carrier; preferably, 10 to 70 wt%, more preferably 20 to 60 wt%, and even more preferably about 40 wt%.

The hydrophilic phase can comprise 10 to 50 wt% of the carrier; preferably 15 to 40 wt%, more preferably 20 to 35 wt%, and even more preferably about 30 wt%.

The macrolide is present in roughly an amount from 1 to 15 wt% of the composition, more preferably about 2 to 10 wt%.

The macrolide can be rapamycin or an O-substituted derivative in which the hydroxy in position 40 of the aforementioned formula is replaced by $-OR_1$, in which R_1 is hydroxyalkyl,

hydroalkoxyalkyl, acylaminoalkyl and aminoalkyl; e.g., 40-O-(2-hydroxy)ethylrapamycin, 40-O-(3-hydroxy)propylrapamycin, 40-O-[2-(2-hydroxy)ethoxy]ethylrapamycin and 40-O-(2-acetaminoethyl)rapamycin. These O-substituted derivatives can be prepared by reaction of rapamycin (or dihydro- or deoxorapamycin) with an organic radical, which is attached under appropriate reaction conditions to a leaving group (e.g., RX, in which R is the organic radical, desired as the O-substituent, such as an alkyl-, allyl- or benzyl moiety, and X is a leaving group like $CCl_3C(NH)O$ or CF_3SO_3). These states can be acid or neutral states, e.g., in the presence of an acid like trifluoromethanesulfonic acid, camphorsulfonic acid p-toluenesulfonic acid, or their corresponding pyridine or substituted pyridine salts when X is $CCl_3C(NH)O$, or in the presence of a base like pyridine, a substituted pyridine, diisopropylethylamine or pentamethylpiperidine when X is CF_3SO_3.

A preferred compound is 40-O-(2-hydroxy)ethylrapamycin (hereafter, compound A), as described in PCT/EP/93/02604.

The aforementioned are examples of compounds of the FK506 class. They include, e.g., FK506, ascomycin and other naturally occurring compounds. They also include synthetic analogues.

A preferred compound of the FK506 class is described in EP 427680, e.g., Example 66a.

Other preferred compounds are described in EP 465426.

The hydrophilic phase can be chosen from Transcutol (which has the formula C_2H_3-[O-$(CH_2)_2$]$_2$-OH), glycofurol (also known as tetrahydrofurfuryl alcohol polyethylene glycol ether) and 1,2-propylene glycol or their mixtures, and is preferably 1,2-propylene glycol. The hydrophilic phase can include additional hydrophilic co-components, e.g., lower alcohols like ethanol. These co-components are generally present as a partial replacement of other components of the hydrophilic phase. It has been found that the use of ethanol, although not essential in the compositions, is a particular advantage if the compositions are to be produced in a soft encapsulated gelatin form. This is due to the fact that the storage features are improved, especially in that the risk of rapamycin precipitation after the encapsulation process is reduced. The storage life can therefore be lengthened by using ethanol or another such co-component as an additional ingredient of the hydrophilic phase. The ethanol can comprise 0 to 60 wt% of the hydrophilic phase, preferably 20 to 55 wt%, and more preferably about 40 to 50 wt%. Small amounts of liquid polyethylene glycols can also be incorporated in the hydrophilic phase.

Preferred lipophilic phase components are triglycerides of medium-length fatty acid chains, mixed mono-, di-, triglycerides and transesterified ethoxylated vegetable oils. Appropriate medium fatty acid triglycerides are those known and available commercially under the trade names Myglyol, Captex, Myritol, Capmul, Captex, Neobee and Mazol; Myglyol 812 is the most preferred. The triglycerides are described in Fiedler, H. P. "Encyclopedia of Excipients

for Pharmacy, Cosmetics and Related Areas," Cantor Edition, D-7960 Aulendorf, Third revised and expanded edition (1989), content of which is incorporated here by reference.

The mixed mono-, di- and triglycerides preferably comprise mixtures of C_{12-20} fatty acid mono-, di-, triglycerides, especially mixed C_{16-18} fatty acid mono-, di- and triglycerides. The fatty acid components of the mixed mono-, di- and triglycerides can include both saturated and unsaturated fatty acid residues. However, they preferably comprise mostly unsaturated fatty acid residues; particularly unsaturated C_{18} fatty acid residues. The mixed mono-, di-, triglycerides appropriately comprise at least 60 wt%, preferably at least 75 wt%, more preferably at least 85 wt% of an unsaturated C_{18} fatty acid (e.g., linolenic, linoleic and oleic acid) mono-, di- and triglycerides. The mixed mono-, di- and triglycerides comprise less than 20 wt%, e.g., 15 wt% or 10 wt% or less, saturated fatty acid (e.g., palmitic and stearic acid) mono-, di- and triglycerides.

The mixed mono-, di- and triglycerides preferably include mostly mono- and diglycerides. For example, mono- and diglycerides comprise at least 50%, more preferably at least 70%, based on the total weight of the lipophilic phase. The mono- and diglycerides more preferably comprise at least 75 wt% (e.g., about 80 wt% or 85 wt%) of the lipophilic phase.

The monoglycerides preferably make up about 25 to about 50% of the mixed mono-, di- and triglycerides, based on the total weight of the lipophilic phase. More preferably, about 30 to about 40% (e.g., 35 to 40%) monoglycerides are present.

The diglycerides preferably make up from about 30 to 60% of the mixed mono-, di- and triglycerides, based on the total weight of the lipophilic phase. More preferably, about 40 to about 55% (e.g., 48 to 50%) diglycerides are present.

The triglycerides appropriately make up at least 5%, but less than about 25% of the mixed mono-, di- and triglycerides, based on the total weight of the lipophilic phase. More preferably, about 7.5 to about 15% (e.g., 9 to 12%) triglycerides are present.

The mixed mono-, di- and triglycerides can be prepared by mixing of individual mono-, di- and triglycerides in appropriate relative percentages. However, in practice, they include transesterification products of vegetable oils, e.g., almond oil, ground nut oil, olive oil, persica oil, palm oil, or preferably corn oil, sunflower oil or safflower oil, and especially corn oil with glycerol.

Such transesterification products are generally obtained by heating the selected vegetable oil with glycerol at high temperature in the presence of an appropriate catalyst in an inert atmosphere with continuous mixing (e.g., in a stainless steel reactor), in order to produce transesterification or glycerolysis. The transesterification products generally additionally include limited amounts of free glycerol in addition to their mono-, di- and triglyceride components. The amount of free glycerol is preferably less than 10%, more preferably less than 5%, and most

preferably about 1 or 2 wt%, based on the total weight of the glycerol plus mono-, di- and triglycerides.

Initially, a small amount of the glycerol is preferably removed in order to produce an "essentially glycerol-free charge," if soft gelatin capsules are to be produced.

Transesterification products of corn oil and glycerol yield particularly suitable mixed mono-, di- and triglycerides. An example of an appropriately mixed glyceride product is the transesterification product available commercially under the trade name MAISINE. This product includes mostly linoleic and oleic acid mono-, di- and triglycerides, together with small amounts of palmitic and stearic acid mono-, di- and triglycerides (corn oil itself comprises ingredients of about 56 wt% linoleic acid, 30% oleic acid, about 10% palmitic acid and about 3% stearic acid). The physical characteristics of MAISINE (available from Etablissement Gattefossé 36, Chemin de Genas, P.O. Box 603, 69804 Saint-Priest, Cedex (France)) are as follows: up to 10% (typically 3.9 to 4.9%, or in "essentially glycerol-free" charges, about 0.2%) free glycerol; about 35% (typically 30 to 40%, or in "essentially glycerol-free" charges, about 32 to 36%, e.g., about 36%) monoglyceride; about 50% (or in "essentially glycerol-free" charges, about 46 to 48%) diglyceride; about 10 (or in "essentially glycerol-free" charges, about 12 to 15%) triglyceride; and about 1% free oleic acid.

Additional physical features for MAISINE are as follows: a maximum acid value of about 2, an iodine number of about 85 to 105, a saponification number of about 150 to 175 (Fiedler "Encyclopedia of Excipients," Third revised and expanded edition, 1989, Vol. 2, p. 768). The fatty acid content for MAISINE is typically as follows: about 11% palmitic acid, about 2.5% stearic acid, about 29% oleic acid, about 56% linoleic acid, and 1.5% other acids.

It is particularly preferred that the mixed mono-, di- and triglycerides are clear and remain clear longer than 20 days during storage at temperatures from 20°C to 25°C. The sample of the mixed mono-, di- and triglycerides that was stored in a refrigerator at about 2 and 8°C for 24 h and then stored at room temperature for 1 h, should also be clear.

The mono-, di- and triglycerides preferably have a low content of saturated fat. Mixed mono-, di- and triglycerides that satisfy these requirements can be obtained from commercially available products by separation methods known in the prior art (e.g., freezing methods connected with separation methods, like centrifuging), in order to remove the saturated fatty acid components and improve the unsaturated fatty acid component content. Typically, the total saturated fatty acid component content will be less than 15 wt% (e.g., <10 wt% or <5 wt%), based on the total weight of the lipophilic phase. A reduction of the content of saturated fatty acid components in the monoglyceride fraction can be observed after it has been subjected to the separation method. An appropriate method is described in WO 93/09211.

The mixed mono-, di- and triglycerides therefore preferably contain smaller amounts of saturated fatty acids (e.g., palmitic and stearic acids) and relatively higher amounts of unsaturated fatty acids (e.g., oleic and linoleic acids) as starting material.

An appropriate example of a mixed mono-, di- and triglyceride product that contains small amounts of saturated fatty acids contains the following: 32 to 36 wt% monoglycerides, 45 to 55% diglycerides and 12 to 20 wt% triglycerides, based on the total weight of the lipophilic phase. Additional characteristics include the following:

(1) Fettsäurengehalt (wie als der Methylester durch Chromatographie bestimmt)	(9) Methyllinoelat 53 bis 63& Mtehyloleat 24 bis 34% Methyllinolenat 0 bis 3& Methylarachat 0 bis 3% Methylpamitat 6 bis 12% Methylstearat 1 bis 3%
(2) relative Dichte	0,94 bis 0,96
(3) Hydroxylzahl	(10) 140 bis 210
(4) Jodzahl	110 bis 20
(5) Peroxidzahl	<4,0
(6) Freies Glycerol	<1,0
(7) Verseifungszahl	ungefähr 150 bis 185 (11)
(8) Säurenwert	max. ungefähr 2 (12)

Key:
1 Fatty acid content (determined as methyl ester by chromatography)
2 Relative density
3 Hydroxyl number
4 Iodine number
5 Peroxide number
6 Free glycerols
7 Saponification number
8 Acid value
9 Methyl linoleate 53 to 63[%]
Methyl oleate 24 to 34%
Methyl linolenate 0 to 3[%]
Methyl arachidate 0 to 3%
Methyl palmitate 6 to 12%
Methyl stearate 1 to 3%
10 ___ to ___
11 about 150 to 185
12 max. about 2

Mixed mono-, di- and triglycerides that meet these characteristics are referred to in this description as "refined glycerol-transesterified corn oils." The "refined glycerol-transesterified corn oils" have the advantage that they remain stable for a long time.

The lipophilic phase, on the other hand, can include appropriate transesterified ethoxylated vegetable oils like those obtained by reaction of different natural plant oils (e.g., corn oil, germ oil, almond oil, ground nut oil, olive oil, soybean oil, sunflower oil, safflower oil and palm oil or mixtures thereof) with polyethylene glycols having an average molecular weight from 200 to 800 in the presence of an appropriate catalyst. These methods are known and an example is described in U.S. Patent No. 3,288,824. Transesterified ethoxylated corn oils particularly preferred.

Transesterified ethoxylated vegetable oils are known and commercially available under the trade name LABRAFIL (H. Fiedler, loc. cit., Vol. 2, p. 707). Examples are LABRAFIL M 2125 CS (which is obtained from corn oil and has an acid number less than about 2, saponification number from 155 to 175, an HLB value of 3 to 4 and an iodine number from 90 to 110) and LABRAFIL M 1944 CS (which is obtained from corn oil and has an acid number of about 2, a saponification number from 145 to 175 and an iodine number from 60 to 90). LABRAFIL M 2130 CS (which is a transesterification product of a C_{12-18} glyceride and polyethylene glycol and has a melting point of about 35 to 40°C, an acid number less than about 2, a saponification number of 185 to 200, and an iodine number less than about 3) can also be used. The preferred transesterified ethoxylated vegetable oil is LABRAFIL M 2125 CS, which can be obtained, e.g., from Gattefossé, Saint-Priest, Cedex, France.

Examples of appropriate surfactants are the following:

i) Reaction products of natural or hydrogenated castor oil and ethylene oxide. The natural or hydrogenated castor oil can react with ethylene oxide in a molar ratio of about 1:35 to about 1:60, with optional removal of the polyethylene glycol component from the products. Different such surfactants are commercially available. The polyethylene glycol-hydrogenated castor oils available under the trade name CREMOPHOR are particularly suitable. CREMOPHOR RH 40, which has a saponification number of about 50 to 60, an acid number less than about 1, a water content (Fischer) less than about 2%, an n_D^{60} of about 1.453 to 1.457 and an HLB value of about 14 to 16 is particularly suitable; and CREMOPHOR RH 60, which has a saponification number of about 40 to 50, an acid number less than about 1, an iodine number less than about 1, a water content (Fischer) of about 4.5 to 5.5%, an n_D^{25} of about 1.453 to 1.457 and an HLB value of about 15 to 17. A particularly preferred product of this class is CREMOPHOR RH 40. Polyethylene glycol castor oils available under the trade name CREMOPHOR EL are also suitable, in which CREMOPHOR EL has a molecular weight (by vapor osmometry) of about 1630, a saponification number of about 65 to 70, an acid number of about 2, an iodine number of

about 28 to 32 and n_D^{25} of about 1.471. Similar or identical products that can also be used are available under the trade names NIKKOL (e.g., NIKKOL HCO-40 and NIKKOL HCO-60), MAPEG (e.g., MAPEG CO-40h), INCROCAS (e.g., INCROCAS 40) and TAGAT (e.g., TAGAT RH 40). The surfactants are further described in Fiedler, loc. cit.

ii) Polyoxyethylene sorbitan fatty acid esters, e.g., mono- and trilauryl, palmityl, stearyl and oleyl esters, of the type available commercially under the trade name TWEEN (Fiedler, loc. cit., pp. 1300-1304), including the products TWEEN

20[polyoxyethylene(20)sorbitan monolaurate],

21[polyoxyethylene(4)sorbitan monolaurate],

40[polyoxyethylene(20)sorbitan monopalmitate],

60[polyoxyethylene(20)sorbitan monostearate],

65[polyoxyethylene(20)sorbitan tristearate],

80[polyoxyethylene(20)sorbitan monooleate],

81[polyoxyethylene(5)sorbitan monooleate],

85[polyoxyethylene(20)sorbitan trioleate].

Particularly preferred products of this class are TWEEN 40 and TWEEN 80.

iii) Polyoxyethylene fatty acid esters, e.g., polyoxyethylene stearic acid esters of the type known and available commercially under the trade name MYRJ (Fiedler, loc. cit., 2, pp. 834-835). A particularly preferred product of this class if MYRJ 52 with a D^{25} of about 1.1, a melting point of about 40 to 44°C, an HLB value of about 16.9, an acid value of about 0 to 1 and a saponification number of about 25 to 35.

iv) Polyoxyethylene-polyoxypropylene copolymers and block copolymers, e.g., of the type known and available under the trade name PLURONIC, EMKALYX and POLOXAMER (Fiedler, loc. cit., 2, p. 959). A particularly preferred product of this class is PLURONIC F68, with a melting point of about 52°C and a molecular weight of about 6800 to 8975. Another preferred product of this class is POLOXAMER 188.

v) Dioctyl sulfosuccinate or di-[2-ethylhexyl] succinate (Fiedler, loc. cit., 1, pp. 107-108).

vi) Phospholipids, especially lecithins (Fiedler, loc. cit., 2, pp. 943-944).

Appropriate lecithins include especially soy lecithins.

vii) Propylene glycol mono- and -difatty acid esters like propylene glycol dicaprylate (also known and available commercially under the trade name MIGLYOL 840), propylene glycol dilaurate, propylene glycol hydroxystearate, propylene glycol isostearate, propylene glycol laurate, propylene glycol ricinoleate, propylene glycol stearate, etc. (Fiedler, loc. cit., 2, pp. 808-809).

It will also be apparent that the components of this carrier can contain unreacted starting materials, e.g., polyethylene glycol.

The selected surfactant preferably has an HLB of at least 10.

The relative proportion of hydrophilic phase component(s) of the lipophilic phase and the surfactant lie within the "microemulsion" range on a normal three-way diagram. The composition so obtained by microemulsion preconcentrates with high stability that are capable of yielding microemulsions after addition of water, which have an average particle size of <1500 Å and are stable over periods of 24 h.

The microemulsion preconcentrate compositions exhibit good stability features, as demonstrated by normal stability experiments, e.g., with a storage life of up to 3 years and even longer.

On the other hand, the components can be selected in order to yield an emulsion preconcentrate. The emulsion preconcentrate compositions also exhibit good stability features as demonstrated by normal stability experiments, e.g., long-term stability of up to 3 years and even longer.

The pharmaceutical composition can also include additional additives or ingredients, e.g., antioxidants (like ascorbyl palmitate, butylhydroxyanisole (BHA), butylhydroxytoluene (BHT) and tocopherols), and/or preservatives. These additives or ingredients can comprise about 0.05 to 1 wt% of the total weight of the composition. The pharmaceutical composition can also contain sweeteners or flavorings in an amount of up to 2.5 or 5 wt%, based on the total weight of the composition. The antioxidant is preferably α-tocopherol (vitamin E).

The pharmaceutical composition can also include one or more immunosuppressants like a cyclosporin, or, if a rapamycin is present, an FK506 compound as described above. Cyclosporins comprise a class of cyclic poly-N-methylated undecapeptides, which generally possess immunosuppressant, anti-inflammatory, anti-viral and/or anti-parasitic activity, each to a greater or lesser degree. The first cyclosporin to be identified was the fungal metabolite cyclosporin A, or cyclosporin, and its structure is provided in the Merck Index, 11th Edition, Merck & Co., Inc., Rahway, New Jersey, USA (1989), under listing 2759. Later cyclosporins were identified are cyclosporins B, C, D and G, which are also listed in Merck Index under listing 2759. A large number of synthetic analogues are also known, and representative examples are described in EP 296122, EP 484281 and GB 2222770. These compounds are referred to collectively in the description as "cyclosporins."

The pharmaceutical composition exhibits particularly advantageous properties if administered orally; e.g., in terms of stability and high degree of bioavailability, which was obtained in normal bioavailability experiments, up to 2 to 4 times higher than emulsions. These experiments are conducted on animals or healthy volunteers, using HPLC or a specific or nonspecific monoclonal layout in order to determine the state of the macrolide in the blood. For example, in the test described in Example 3, 10 mg rapamycin was administered p.o. to rats and

surprisingly high C_{max} values between 2670 and 3400 ng/mL were found by ELISA using a specific monoclonal antibody. It was also found that an emulsion preconcentrate and a microemulsion preconcentrate composition have much better pharmacokinetic properties than a normal solvent system.

Pharmacokinetic parameters, like absorption and blood levels, are also surprisingly easily predictable and problems of administration with incalculable absorption can be eliminated or reduced. In addition, the pharmaceutical composition with surfactants is effective; for example, bile salts that are present in the gastrointestinal tract. This means the pharmaceutical composition is fully soluble in aqueous systems that include such natural surfactants and can therefore deliver microemulsion systems in situ that are stable and do not exhibit precipitation of the active ingredient or other disturbance of fine particle structure. The function of the pharmaceutical composition after oral administration essentially remains independent and/or uninfluenced from the relative presence or absence of bile salts at any specific time or for any specific person.

The pharmaceutical composition is preferably bound in a unit dosage configuration, e.g., by its filling into orally administerable capsule shells. The capsule shells can be soft or hard gelatin capsule shells. Where the pharmaceutical composition has a unit dose configuration, each unit dose will appropriately contain between 10 and 100 mg of the macrolide, more preferably between 10 and 50 mg; e.g., 15, 20, 25 or 50 mg of the macrolide. Such unit dose configurations are suitable for administration from 1 to 5 times a day depending on the specific therapeutic objective, the phase of therapy, etc.

If desired, the pharmaceutical composition, however, can also be in a drinkable formulation and can include water or any other aqueous system in order to furnish emulsion or microemulsion systems suitable for drinking.

The use of the pharmaceutical composition can be observed in normal clinical tests in known indications of macrolide dosages that give equivalent blood levels of macrolides: e.g., use of dosages in the range from 2.5 to 1000 mg of macrolide per day for an adult weighing 75 kg, and in normal animal models. The increased bioavailability delivered by the compositions of the active ingredient can be observed in normal animal experiments and in clinical experiments. If a cyclosporin or FK506 compound is included in the pharmaceutical composition, this usefulness can also be observed in normal clinical tests and animal models. The macrolide doses that should be used in clinical tests are the ones stated above, whereas those for cyclosporin can be in the range from 25 mg to 1000 mg per day, and those for an FK506 compound 2.5 mg to 1000 mg per day for an adult weighing 75 kg.

The optimal macrolide dose that should be administered to a specific patient must be considered carefully by the treating physician as an individual reaction to the metabolism of rapamycin, since the metabolism of the rapamycin compound can vary. It can be advisable to

monitor the blood serum level of the rapamycin compound by radioimmunoassay, assay of monoclonal antibodies or other appropriate conventional means. Macrolide doses are generally from 2.5 mg to 1000 mg per day for an adult weighing 75 kg, preferably 25 mg to 500 mg, in which the optimal dose is 50 to 100 mg per day. Satisfactory results are achieved by administering about 75 mg per day, e.g., in the form of two capsules, one containing 50 mg and one containing 25 mg; or three capsules, each containing 25 mg. If a cyclosporin or FK506 compound is included in the pharmaceutical compositions, the cyclosporin dose can be 25 to 1000 mg per day (preferably 50 to 500 mg), and the FK506 compound dose can be 2.5 mg to 1000 mg per day (preferably 10 mg to 250 mg).

The pharmaceutical compounds are particularly useful for the following states:

a) Treatment and prevention of organ transplant rejection, e.g., for treatment of recipients of heart, lung, combined heart–lung, liver, kidney, pancreas, skin or corneal transplants. The pharmaceutical compositions are also indicated for preventing graft-versus-host disease, which sometimes occurs after bone marrow transplants.

b) Treatment and prevention of autoimmune diseases and inflammatory states, especially inflammatory states with an etiology that includes an autoimmune component, like arthritis (e.g., rheumatoid arthritis, chronic progressive arthritis and arthritis deformans) and rheumatic diseases. Specific autoimmune diseases for which the pharmaceutical compositions can be used include autoimmune hematological diseases (including hematological anemia, aplastic anemia, anemia of red blood cells and idiopathic thrombocytopenia), systemic lupus erythrematosus, polychondritis, scleroderma, Wegener's granulomatosis, dermatomyositis, chronic active hepatitis, myasthenia gravis, psoriasis, Steven-Johnson syndrome, idiopathic sprue, autoimmune inflammatory intestinal disease (including ulcerative colitis and Crohn's disease), endocrine opthalmopathy, Greaves disease, sarcoidosis, multiple sclerosis, primary biliary cirrhosis, juvenile diabetes (diabetes mellitus type I), uveitis (anterior and posterior), keratoconjunctivitis sicca and vernal keratoconjunctivitis, interstitial pulmonary fibrosis, psoriatic arthritis, glomerulonephritis (with and without nephrotic syndrome, e.g., including idiopathic nephrotic syndrome or nephropathy of minimal alteration) and juvenile dermatomyositis.

c) Treatment and prevention of asthma.

d) Treatment of multidrug resistance (MDR = resistance against a number of chemical cytostatics). The rapamycin compounds suppress P-glycoproteins (Pgp), which are membrane transport molecules connected with MDR. MDR is particularly problematical in cancer patients and AIDS patients, who do not react to conventional chemotherapy, since the medication is pumped from the cells by Pgp. The pharmaceutical compounds are therefore useful, in order to improve the efficacy of other chemotherapeutic agents in the treatment and control of multidrug resistant states, like multidrug resistant cancer or AIDS.

The rapamycin compounds also exhibit anti-tumor and anti-fungal activity and the pharmaceutical compositions can therefore be used as anti-tumor and anti-fungal agents.

In another viewpoint, the invention also provides a method for preparation of a pharmaceutical composition as defined above in which the method includes (1) a hydrophilic phase; (2) a lipophilic phase and (3) a surfactant in an intimate mixture, and adding the compound of the macrolide class. If desired, the composition can be combined in a unit dose configuration, e.g., filling gelatin capsules with the composition.

Additional components or additives can optionally be mixed, especially a co-component of the hydrophilic phase, e.g., ethanol, with components (1), (2) and (3), or with or after addition of the macrolide.

The composition can be combined with sufficient water or a sufficient amount of an aqueous solvent so that a microemulsion or emulsion is obtained.

The contents of all reference numbers referred to above, especially the example compounds, are included here by reference, and each of the example compounds can be used as a macrolide in the examples listed below.

The following examples represent compositions in unit dose configuration that are suitable for use in, e.g., the prevention of transplant rejection or in the treatment of autoimmune disease, during administration from 1 to 5 unit doses/day. The examples are described with particular reference to rapamycin, but equivalent compositions can be obtained that use a different macrolide.

Example 1: Refined corn oil, transesterified with glycerol, is prepared as follows

Corn oil transesterified with glycerol, which is essentially glycerol-free, is slowly cooled to a temperature of +20°C and stored overnight at this temperature. The corn oil is centrifuged at an acceleration of 12,000 G and a flow rate of 103 kg/h in a continuous flow centrifuge in order to produce a liquid phase (62 kg/h) and a phase containing a sediment (41 kg/h). The liquid phase is slowly cooled to +8°C and stored overnight at this temperature. The liquid phase is then centrifuged at an acceleration of 12,000 G and a flow rate of 112 kg/h in order to produce a liquid phase (76.2 kg/h) and a phase containing sediment (35.8 kg/h). The liquid phase is "refined, glycerol-transesterified corn oil." On the other hand, an improved product can be obtained by centrifuging in three steps, e.g., at +20°C, +10°C and +5°C.

The method is characterized by a low percentage reduction in the monoglyceride component in the refined corn oil transesterified with glycerol, compared with the starting material (e.g., 35.6%, in comparison with 38.3%).

Example 2

The refined corn oil transesterified with glycerol described in Example 1 is used to prepare the following oral unit dose configuration:

(1) KOMPONENTE	(2) MENGE (mg/Kapsel)
Rapamycin	20,0
1) Ethanol	75,0
2) 1,2-propylene glycol	81,0
3) refined oil	121,5
4) Cremophor RH40	202,5
Total	500,0

Key: 1 Component
2 Amount (mg/capsule)

The rapamycin is suspended in (1) with agitation at room temperature, and (2), (3) and (4) are added during agitation to the obtained solution. Hard gelatin capsules of size 0 are filled with the mixture obtained and sealed using the quasi-seal process.

Example 3: Pharmacokinetics

Two formulations, prepared as in Example 2 are used:

(1) Formulierung	(2) Komponente	(3) Menge
A	Tween 80	41,5%
	Maisine	24,9%
	Propylene	16,6%
	Ethanol	15,0%
	Rapamycin	2,0%
B	Cremophor RH40	41,5%
	Maisine	24,9%
	Propylene glycol	16,6%
	Ethanol	15,0%
	Rapamycin	2,0%

Key: 1 Formulation
2 Component
3 Amount

Formulation A is an emulsion preconcentrate and formulation B is a microemulsion preconcentrate. 6 male Wistar rats with a mean body weight of 300 g are used per formulation.

One day before treatment, feed is withdrawn from the rats, but the rats are permitted free access to water. The rats are then anesthetized by intraperitoneal injection of 2 × 1 mL 20% urethane, and a permanent catheter is inserted into the right jugular vein in order to permit blood sampling. 500 mL/animal of the formulation is administered by gastric administration 20 h after the operation. The total dose of 10 mg of the drug per animal is administered. Blood samples of 0.7 mL are taken from the jugular catheter of each animal 15 min before drug administration, and 0.17, 0.5, 1, 1.5, 2, 3, 5 and 8 h after drug administration. The samples are kept in heparinized tubes and analyzed by ELISA using microtiter plates coated with specific antibodies to rapamycin. The animals are sacrificed immediately after taking the last blood sample. The results are shown in the following table:

(1) For m	AUC (0-8 Std)* [ng.h/ml]	CV [%]	C_{max} [ng/ml]	CV [%]	t_{max} (2) [Std]	CV [%]
A	11951	44	2671	42	3,8	29*
B	13826	13	3405	30	4,0	35+

(3) *) n = 5

+) n = 2 wegen Schwierigkeiten bei der Blutabnahme

Key: 1 For m
2 h
3 *) n = 5
+) n = 2, because of difficulties during blood sampling

The results show that rapamycin is well absorbed.

Example 4: Comparison

Formulations A and B are compared with the formulation that includes 38.6% corn oil, 41.6% Labrafil M21/25C, 17.8% ethanol and 2% rapamycin (formulation C). The same procedure as in Example 3 is used, except that the animals each receive a total dose of 0.5 mg of the formulation.

The results are shown in the following table:

(1) For m	AUC(0-8 Std) [ng.h/ml] (2)	CV [%]	C_{max} [ng/ml]	CV [%]	t_{max} (2) [Std]	CV [%]
A	105,8	28	31,22	35	1,6	51*
B	96,6	32	36,13	60	0,4	30
C	36,2	31	7,83	27	3,0	78

*) n = 4.

Key: 1 For m
2 h

The results show that formulations A and B yield much better pharmacokinetic properties than formulation C.

Example 5

An active compound of the FK506 class or the rapamycin class, e.g., compound A, is prepared as a microemulsion preconcentrate with the following composition in wt%: 2% active compound; 44% Cremophor RH40, 26.4% corn oil mono-, -di- and -triglycerides, 17.6% 1,2-propylene glycol and 10% ethanol.

Claims

1. Pharmaceutical composition comprising a macrolide and a carrier that includes a hydrophilic phase, a lipophilic phase and a surfactant.

2. Composition according to Claim 1 in the configuration of an emulsion or microemulsion preconcentrate.

3. Composition according to Claim 1 or 2, in which the lipophilic phase includes 10 to 85 wt% of the carrier.

4. Composition according to Claims 1 to 3, in which the surfactant comprises 5 to 80 wt% of the carrier.

5. Composition according to Claims 1 to 4, in which the hydrophilic phase comprises 10 to 50 wt% of the carrier.

6. Composition according to Claims 1 to 5, in which the compound of the rapamycin class is present as 2 to 15 wt% of the composition.

7. Composition according to Claims 1 to 6, in which the macrolide is a compound of the rapamycin class.

8. Composition according to Claims 1 to 6, in which the macrolide is an FK506 compound.

9. Microemulsion preconcentrate carrier for an orally administerable agent that is different from cyclosporin and includes the following:

i) a reaction product of castor oil and ethylene oxide,

ii) a transesterification product of a vegetable oil or glycerol that contains mostly mono-, di- and triglycerides of linoleic or oleic acid or a polyoxyalkylated vegetable oil,

iii) 1,2-propylene glycol and

iv) ethanol.